

**Cerebrovascular carbon dioxide reactivity and flow mediated dilation in
young healthy South Asian and Caucasian European men**

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Running Title: Ethnic differences in cerebrovascular reactivity

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22 **ABSTRACT**

23 South Asians living in the UK have a 1.5-fold greater risk of ischemic stroke than the
24 general population. Impaired cerebrovascular carbon dioxide (CO₂) reactivity is an
25 independent predictor of ischemic stroke and cardiovascular mortality. We sought to test the
26 hypothesis that cerebrovascular CO₂ reactivity is reduced in South Asians. Middle cerebral
27 artery blood velocity (MCA V_m) was measured at rest and during stepwise changes in partial
28 pressure of end-tidal CO₂ (P_{ET}CO₂) in South Asian (n=16) and Caucasian European (n=18)
29 men that were, young (~20 years), healthy and living in the UK. Incremental hypercapnia
30 was delivered via the open circuit steady-state method, with stages of 4% and 7% CO₂ (~21%
31 Oxygen, Nitrogen balanced). Cerebrovascular CO₂ reactivity was calculated as the change in
32 MCA V_m per mmHg change in P_{ET}CO₂. MCA V_m was not different in South Asian (59 (9)
33 cm/s; mean (standard deviation)) and Caucasian Europeans (61 (12) cm/s; P>0.05). Similarly,
34 cerebrovascular CO₂ reactivity was not different between the groups (South Asian, 2.53
35 (0.76) cm/s/mmHg vs. Caucasian European, 2.61 (0.81) cm/s/mmHg; P>0.05). Brachial
36 artery flow-mediated dilatation was lower in South Asian (5.48 (2.94) %) compared to
37 Caucasian European (7.41 (2.28) %; P<0.05); however when corrected for shear rate, no
38 between group differences in flow-mediated dilatation were observed (P>0.05). Flow-
39 mediated dilation was not correlated with cerebrovascular CO₂ reactivity measures. In
40 summary, cerebrovascular CO₂ reactivity and flow-mediated dilation when corrected for
41 shear rate are preserved in young healthy South Asian men living in the UK.

42

43 **Keywords:** brain, cerebral circulation, flow-mediated dilatation.

44 **NEW AND NOTEWORTHY**

45 Previous reports have identified an increased risk of ischemic stroke and peripheral
46 endothelial dysfunction in South Asians compared to Caucasian Europeans. The main finding
47 of this study is that cerebrovascular carbon dioxide reactivity (an independent predictor of
48 ischemic stroke) is not different in healthy young South Asian and Caucasian European adult
49 men.

50

51 **ABBREVIATIONS**

52 BP, blood pressure; CO₂, carbon dioxide; CVCi, cerebrovascular conductance index;
53 ECG, electrocardiograph; FMD, flow-mediated dilation; FMDc, covariate corrected flow-
54 mediated dilation; HR, heart rate; MAP, mean arterial pressure; MCA_V, middle cerebral
55 artery mean blood velocity; N₂, nitrogen; O₂, oxygen; P_{ET}CO₂, partial pressure of end-tidal
56 carbon dioxide; SR_{AUC}, shear rate area under the curve; TCD, transcranial Doppler

57 **INTRODUCTION**

58 South Asian migrants from the Indian sub-continent in the United Kingdom have an
59 ischemic stroke mortality that is ~1.5 times greater than the general population (44), while
60 ischemic stroke onset typically occurs at a younger age in South Asians than ethnically White
61 Caucasian Europeans (20). Although broadly attributable to cultural and socioeconomic
62 factors (12), there is a paucity of information about the underlying physiological mechanisms
63 for such ethnic differences in cerebrovascular health (37). The brain has a high metabolic
64 demand and possesses multiple interactive regulatory mechanisms. The latter ensure that
65 cerebral blood flow remains relatively stable independent of changes in perfusion pressure
66 (cerebral autoregulation), that local perfusion is closely matched to neuronal activation and
67 metabolism (neurovascular coupling), and that cerebrovascular responses to changes in
68 carbon dioxide (cerebrovascular CO₂ reactivity) are adequate to assist the maintenance of
69 central [H⁺]. Bathula et al. (4) observed that cerebral autoregulation is poorer and
70 cerebrovascular resistance is higher in South Asians (of Punjabi Sikh origin) compared to
71 people with “European origins”. However, it remains to be determined whether
72 cerebrovascular CO₂ reactivity is blunted (i.e., diminished cerebral vasodilatory reserve) in
73 South Asians.

74 It has long been established that the cerebral vasculature is highly sensitive to changes
75 in the partial pressure of arterial CO₂ (19), and since this time an impaired cerebrovascular
76 CO₂ reactivity has been established as an independent predictor of ischemic stroke (23) and
77 identified in several cardiovascular, cerebrovascular and neurological disorders (13, 18, 23,
78 41). Cerebrovascular dysfunction may lead to neuronal dysfunction and neurodegeneration
79 since neurons depend on arterial vasodilatation for adequate perfusion to ensure oxygen/CO₂
80 homeostasis, nutrient delivery and elimination of potentially toxic metabolites (46). The
81 mechanism whereby CO₂ modifies cerebral blood vessel tone is complex. Among the various

82 contributory factors, endothelium-derived nitric oxide is considered to be an important local
83 regulator of cerebral blood flow that plays a role in hypercapnia-induced vasodilatation (14,
84 40, 43). Indeed, acute infusion of L-arginine (the substrate for endothelial nitric oxide
85 synthase) restores impairments in cerebrovascular CO₂ reactivity manifest in patients with
86 cardiovascular risk factors (45), while hypercapnia-induced increases in cerebral blood flow
87 are attenuated by inhibition of nitric oxide synthase activity with N-nitro-L-arginine methyl
88 ester (L-NAME) in rats (5). Moreover, individuals or groups in whom impaired peripheral
89 vascular nitric oxide signaling has been identified are reported to demonstrate a reduced
90 cerebrovascular CO₂ reactivity (21). Therefore, the observation that brachial artery flow-
91 mediated dilation, indicative of attenuated endothelium-derived nitric oxide mediated
92 vasodilation, is reduced in South Asians compared to Caucasian Europeans (6, 30) may also
93 point to a reduced cerebrovascular CO₂ reactivity.

94 The aim of this study was to investigate whether cerebrovascular CO₂ reactivity is
95 impaired in young healthy South Asians compared to Caucasian Europeans. Based on prior
96 reports identifying the greater incidence of cerebrovascular events in South Asians and
97 peripheral endothelial dysfunction, we hypothesized that cerebrovascular CO₂ reactivity
98 would be lower in healthy young South Asian adults when compared to age-matched
99 Caucasian Europeans. Brachial artery flow-mediated dilation, a well-established marker of
100 peripheral vascular (endothelial) function, was also determined in accordance with
101 established guidelines (32, 42). Lastly, we assessed whether an association between brachial
102 artery flow-mediated dilation and cerebrovascular CO₂ reactivity existed in the population
103 studied.

METHODS

Ethical Approval.

The experiments were undertaken in accordance with the Declaration of Helsinki, except for registration in a database, and were approved by the University of Birmingham, Science, Technology, Engineering and Mathematics Ethical Review (approval number ERN_17-1161). Written informed consent was obtained from all participants after each had received a detailed verbal and written explanation of the study procedures.

Participant characteristics.

Sixteen South Asians with ethnic roots in Indian-Subcontinent (Bangladesh, India, Maldives, Nepal, Pakistan and Sri Lanka) and eighteen Caucasian Europeans living in the UK volunteered for this study. Accordingly, each participant confirmed the ethnic origins of all four of their grandparents. South Asian participants were first or second-generation migrants. No participant had a known history of pulmonary, cardiovascular, metabolic or neurological diseases and were not taking prescription or over-the-counter medication. One participant in each group was found to have raised blood pressure and recommended to have an appointment with their general practitioner. Upon follow-up both were confirmed as being normotensive. All participants were accustomed to recreational exercise, but none was a competitive athlete.

Experimental measures.

Height and weight, along with waist (level of the umbilicus) and hip (level of the femoral trochanter) circumference were measured. Heart rate (HR) was monitored using a lead II electrocardiogram (ECG) (Morgan 509 Cardiac Monitor, Kent, UK). Arterial blood pressure (BP) was measured continuously using finger photoplethysmography (Portpress,

Finapres Medical Systems BV, Amsterdam, The Netherlands) and corrected with automatic brachial sphygmomanometer readings (Omron 750IT, Milton Keynes, UK). Middle cerebral artery mean blood velocity (MCA V_m) was continuously monitored using transcranial Doppler ultrasonography (Doppler Box X, DWL, Sipplingen, Germany). A 2 MHz probe, mounted on an adjustable headband, was fixed at the temporal window to insonate the right MCA at a depth of 40-65 mm. Participants wore a mouthpiece and nose-clip, and the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) was provided by a capnograph connected to the mouthpiece by an anesthetic sample line (Gas Analyzer, ADInstruments, Dunedin, New Zealand). Breath-by-breath fluctuations in $P_{ET}CO_2$ were used to calculate respiratory rate. Analogue signals were digitized at 1 kHz (Powerlab, ADInstruments) and recorded using multi-channel data acquisition software (LabChart 7, ADInstruments). Simultaneous recordings of the left brachial artery diameter and flow velocity were obtained with the arm at heart level using Doppler ultrasound (Terason uSmart 3300, Teratech Corporation, Burlington, MA, USA). The artery was insonated 10–15 cm proximal to the medial epicondyle at 60°. Duplex imaging was used to obtain a B-mode image of vessel diameter and pulse-wave mode of peak blood velocity using a 4-15 Hz multi-frequency linear-array transducer (Terason uSmart 15L4) held in place with an adjustable probe holder. Ultrasound measurements were made in accordance with technical recommendations (32, 42). Recordings were screen captured, stored as video files and offline analysis carried out using automated edge detection and wall tracking software (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy) (11).

Experimental Protocol

This cross-sectional study included a screening/familiarization visit prior to the experimental session. Participants were instructed to abstain from food for 2 h, caffeinated beverages for 12 h, strenuous exercise for 24 h and multi-vitamin use for 7 days before

experimental sessions. The study was conducted in a temperature controlled cardiovascular laboratory (21–24 °C). Participants were asked to lie supine comfortably for ~10 min on a medical examination couch. A narrow inflatable cuff (5 cm width, Hokanson, Bellevue, WA, USA) was placed 5-7 cm distal to the medial epicondyle. The flow-mediated dilatation protocol was then conducted with the brachial artery insonated for the simultaneous measurement of diameter and flow velocity. The flow-mediated dilatation protocol comprised of a 2 min baseline, a 5 min cuff inflation to a supra-systolic pressure of > 240 mmHg and a 3 min recovery period with the cuff deflated.

To assess cerebrovascular CO₂ reactivity a 10-min baseline was acquired while participants breathed room air. During this period, a minimum of 3 brachial artery blood pressure readings were obtained using the automated sphygmomanometer. Participants then breathed gas mixtures from a Douglas bag containing air enriched with CO₂ (hypercapnia), via a two-way non-rebreathing valve. Specifically, participants received 4 % CO₂ (≈21 % O₂, N₂ balanced) for 4-min, followed by 7 % CO₂ (≈21 % O₂, N₂ balanced) for 4 min, then were switched back to room air (18, 33). Hemodynamic and respiratory parameters were recorded throughout and once these had returned to baseline, participants were asked to increase their respiratory depth and rate in order to achieve an equal but opposite change in their P_{ET}CO₂ as during the hypercapnic challenge, with each step lasting 2 min (hypocapnia).

Data analysis

Body mass index (BMI) was expressed as the ratio of the participants' weight and the height squared. Digitally recorded data were extracted in an anonymized manner. Mean arterial pressure (MAP) was the mean blood pressure over each cardiac cycle. Brachial artery blood flow was calculated as:

$$\text{Brachial artery blood flow} = \left[\frac{\text{Peak Envelope Velocity}}{2} \cdot (\pi (0.5 \cdot \text{Diameter})^2) \right] \cdot 60$$

Brachial artery flow-mediated dilatation was taken as the maximal change in brachial artery diameter following cuff deflation. The time to peak diameter was obtained between the cuff deflation and the maximal artery dilation, and time to peak blood flow (reactive hyperemia) was obtained between cuff deflation and maximal flow velocity. Shear rate was calculated as brachial artery blood velocity multiplied by 4 and divided by brachial artery diameter. Shear rate area under the curve (SR_{AUC}) was calculated as an integral between the cuff deflation and the maximal artery dilation. Flow-mediated dilatation was expressed as absolute and relative change in diameter. A ratio between flow-mediated dilatation and SR_{AUC} (FMD-to-SR_{AUC} ratio) was also calculated and multiplied by 1000 (32, 42). Further, based on recent guidelines (2), baseline and maximal brachial artery diameters were log-transformed and the difference between them calculated. Logged difference in diameter was entered in an analysis of covariance (ANCOVA) where ethnicity constituted a fixed factor and log-transformed baseline diameter a covariate. The covariate adjusted means were then back-transformed and expressed as percentage changes for covariate corrected flow-mediated dilatation (FMD_C).

Cerebrovascular conductance index (CVCi) was calculated as $MCA V_m / MAP$. Baseline values are taken as mean of the whole 10-min baseline period. For cerebrovascular CO₂ reactivity, values were acquired over the last minute of each hypercapnic and hypocapnic step. Cerebrovascular CO₂ reactivity was assessed using linear and exponential models (39). For exponential model, values of the exponent and R² and for linear model, the values of slope and R² of % change in (Δ) MCA V_m and % Δ CVCi versus P_{ET}CO₂ (mmHg) were calculated. Cerebrovascular CO₂ reactivity was separately expressed as the linear slope of Δ MCA V_m (cm/s) and Δ CVCi (cm/s/mmHg) versus the change in P_{ET}CO₂ in mmHg, between the two hypercapnic steps and two hypocapnic steps (18, 33). Additional analyses of cerebrovascular CO₂ reactivity were undertaken by calculating the slope of % Δ MCA V_m

and % Δ CVCi versus Δ $P_{ET}CO_2$ (in mmHg) with the hypercapnic and hypocapnic steps (9, 31).

Statistical Analysis

Data distribution was assessed by the Shapiro-Wilk test. Normally distributed data were analyzed using two-tailed Students t-test, while non-normally distributed data were analyzed using Mann-Whitney Rank Sum test. The correlation between cerebrovascular CO_2 reactivity and flow-mediated dilatation was assessed using Pearson's product moment correlation. Effect size (Cohen's d) was calculated as the difference between means of two groups divided by the averaged standard deviation (SD). Statistical analysis was performed using Sigmaplot 13.0 (Systat Software Inc, London, UK). Significance was set at $p < 0.05$. Normally distributed data are presented as mean (SD), unless stated, while non-normally distributed data are presented as median [interquartile range].

RESULTS

Participant characteristics and baseline haemodynamics

Participant characteristics are presented in Table 1. Groups were closely matched for age, weight, BMI and waist-to-hip ratio. At baseline, no between-group differences in heart rate, systolic BP, diastolic BP and respiratory rate were observed. Similarly, MCA V_m , CVCi and MAP were not different between the South Asian and Caucasian European groups ($P>0.05$), however $P_{ET}CO_2$ was lower in South Asians ($P<0.05$; Figure 1).

Cerebrovascular CO_2 reactivity

Figure 2 shows the MCA V_m , CVCi and MAP response to both the hypercapnic and hypocapnic steps of the cerebrovascular CO_2 reactivity test in the South Asian and Caucasian European groups. As anticipated, hypercapnia produced pronounced increases in MCA V_m and CVCi, while conversely both were reduced with hypocapnia. Of note, no between-group differences were observed in any index of cerebrovascular CO_2 reactivity (Figure 3, Table 2).

Brachial artery flow-mediated dilatation

Flow-mediated dilatation was lower in the South Asian than Caucasian European group ($P<0.05$, Figure 4). This between group difference persisted with correction for baseline diameter (FMD_C $P<0.05$, Table 3). Peak reactive hyperemia was not different between groups ($P>0.05$, Table 3). However, SR_{AUC} was lower in South Asians than Caucasian Europeans ($P<0.05$, Table 3) and when brachial artery flow-mediated dilatation was corrected for SR_{AUC} (i.e., FMD-to-SR_{AUC} ratio) the between group difference was no longer evident ($P>0.05$, Figure 4). No significant association between FMD_C and hypercapnic cerebrovascular CO_2 reactivity (4% to 7%; Figure 3) was observed either for the whole group

240 (r = 0.08, P = 0.669), or individually for South Asians and Caucasian Europeans (r = -0.05, P
241 = 0.854 and r = 0.18, P = 0.475, respectively).

DISCUSSION

The major novel finding of the present study is that cerebrovascular CO₂ reactivity is not different in young healthy South Asians and Caucasian Europeans. In addition, brachial artery flow-mediated dilatation was lower in South Asians when expressed as a percentage change from baseline. However, during flow-mediated dilation testing South Asians had a lower shear rate response (SR_{AUC}), which when accounted for (FMD-to-SR_{AUC} ratio), flow-mediated dilatation was not different between groups. These findings suggest that: 1) contrary to our hypothesis, cerebrovascular CO₂ reactivity is not lower in healthy young South Asian adults than age-matched Caucasian Europeans, and 2) apparent reductions in brachial artery flow-mediated dilatation in South Asians (6, 30) may be attributable to a reduced ischemic stimulus rather than endothelial dysfunction *per se*.

Prior reports have identified a greater incidence of cerebrovascular events in South Asians (20, 44). Given the prognostic significance of impaired cerebrovascular CO₂ reactivity as an independent predictor of ischemic stroke (23) and its association with multiple cardiovascular, cerebrovascular and neurological disorders (13, 18, 23, 41), we anticipated that cerebrovascular CO₂ reactivity would be lower in South Asian adults than age-matched Caucasian Europeans. Moreover, Hurr et al. (15) identified that African Americans (23±4 years), a group at higher risk of cardiovascular and cerebrovascular disease, exhibited an attenuated cerebrovascular vasodilatation in response to hypercapnia compared to age-matched Caucasian Americans. Contrary to expectation, we did not observe a difference in cerebrovascular CO₂ reactivity between young healthy South Asian and Caucasian European men; neither did we observe between-group differences in MCA V_m nor CVCi. In a population-based sample Bathula et al. (4) noted a higher MCA V_m (38.0±0.7 vs. 41.4±0.7 cm/s) and cerebrovascular resistance (resistivity index), but poorer cerebral autoregulation (low frequency gain, 0.45±0.01 vs. 0.50±0.01 cm/s/mmHg) in South Asians of Punjabi Sikh

origin (n=127) compared to people with “European origins” (n=128). Interestingly, the elevated cerebrovascular resistance in South Asians was attributable to hyperglycaemia (e.g., blood glucose, glycated haemoglobin). The cohort studied by Bathula et al. (4) had a wide age range (35-75 years) and comorbidities, including hypertension, diabetes, coronary heart disease and metabolic syndrome, which perhaps is reflected in their comparatively low MCA V_m values (7, 17, 18, 29). However, this is in contrast to the young and healthy participants recruited to the present study and may explain why we did not observe any differences in MCA V_m , CVCi and cerebrovascular CO_2 reactivity between the South Asian and Caucasian European groups studied.

Coronary heart disease risk is elevated in migrant South Asians to the UK (3, 25). Of note, according to the 1991 England and Wales Census data, the relative risk of death from coronary heart disease was 3 in Indian Asian men aged 20-29 years, compared to age-matched Caucasian Europeans (3). The excess coronary heart disease risk in South Asians is not explained by conventional risk factors (e.g., smoking, hypercholesterolemia, hypertension) (24), although an increased prevalence of insulin resistance and diabetes has been implicated (26). Endothelial dysfunction in South Asians (i.e., attenuated brachial artery flow-mediated dilatation and N^G -Monomethyl-L-arginine induced vasoconstriction) is also speculated to contribute to the elevated coronary heart disease risk, and has been identified in both young (30) and older (6) South Asian groups. In the present study when we expressed flow-mediated dilatation simply as the percentage change from baseline in brachial artery diameter, it was reduced in South Asians compared to Caucasian Europeans. This experimental approach and the associated findings are in agreement with previous reports (6, 30). It is noteworthy that despite no differences in baseline brachial artery diameter, velocity and blood flow, the SR_{AUC} was attenuated in the South Asian group. Accordingly, when flow-mediated dilatation responses were adjusted to account for this (i.e., via the FMD-to-

SR_{AUC} ratio), the between group difference was no longer observed. This is important because the magnitude of the evoked shear stress is mechanistically coupled with the dilatation observed, but no previous studies reporting a blunted flow-mediated dilatation in South Asians versus European Caucasians have accounted for this (6, 20, 30, 44). In accordance with recent guidelines (32, 42), it is deemed important to account for shear stress when making between group comparisons. The reason for the lower SR_{AUC} in South Asian group is unclear, but may relate to a lower maximal vascular conductance and/or attenuated metabolic vasodilation induced by ischemia. Indeed, as the hyperemia dynamics are coupled with metabolism, it is possible that the results of this study reflect a lower and/or altered metabolic response to ischemia in South Asians; a possibility that requires further investigation.

Brachial artery flow-mediated dilatation and hypercapnia-induced cerebral vasodilatation share common mechanisms, with endothelial derived nitric oxide reported to mediate both, at least partially (14, 16, 40, 43). In the population-based Rotterdam Study, Portegies et al. (36) observed that lower cerebrovascular CO₂ reactivity was associated with an increased risk of all-cause mortality (1.10, 95% confidence interval [CI] 1.01-1.19), cardiovascular mortality (1.09 [95% CI 0.94-1.26]) and non-cardiovascular mortality (1.10 [95% CI 0.99-1.21]), which points towards cerebrovascular CO₂ reactivity being more broadly associated with systemic vascular dysfunction. Moreover, brachial artery endothelial dysfunction (i.e., attenuated forearm reactive hyperemia) and impaired cerebrovascular CO₂ reactivity coexist in patients with long standing diabetes and/or hypertension (21). Similarly, both an impaired cerebrovascular responses to hypercapnia (15) and an attenuated brachial artery flow-mediated dilatation (34) have been identified in African Americans, relative to Caucasian Americans, albeit not in the same cohort. In contrast, we observed no association between cerebrovascular CO₂ reactivity and brachial artery flow-mediated dilatation in our

study population, which possibly reflects the young and healthy cohort with a relatively narrow (i.e., normal) range of vascular responsiveness.

The results of this study should be viewed in the context of the following experimental limitations. Despite the widely acknowledged value of transcranial Doppler in the evaluation of cerebral vascular function, it is an inherent limitation of the method that MCA V_m is only proportional to cerebral blood flow if the cross-sectional area of the MCA remains unchanged. Although, good correlations have been observed between MCA V_m and cerebral blood flow when $P_{ET}CO_2$ is altered (8, 35), there is evidence to suggest MCA diameter increases with robust hypercapnia (i.e., $\Delta P_{ET}CO_2$ of greater than ~ 7 -9 mmHg) (1, 22, 28). $P_{ET}CO_2$ has been employed as a non-invasive surrogate for the partial pressure of arterial CO_2 (P_aCO_2) in the present study because a strong positive linear correlation between $P_{ET}CO_2$ and P_aCO_2 has been identified (27); however, it is acknowledged that $P_{ET}CO_2$ may underestimate P_aCO_2 at rest (38). We also acknowledge the ongoing debate relating to the relative strengths and weaknesses of approaches developed to determine cerebrovascular CO_2 reactivity (10). The method used here has shown a good between-day test-retest reliability (intraclass correlation of 0.938 [95% CI 0.759-0.985] $P < 0.001$; co-efficient of variation for the method error of 6.06%) (18). The extent to which our findings may be more broadly generalized is limited by the inclusion of only healthy young men. We also failed to collect diet and socioeconomic data for the participants and did not objectively assess their activity patterns in a detailed manner. Future studies should consider the important potential interaction between sex, aging, diet, socioeconomic levels, activity patterns and ethnicity in the regulation of peripheral vasculature and cerebrovascular function.

In summary, we report for the first time that cerebrovascular CO_2 reactivity is not different in young healthy South Asians and Caucasian Europeans. Furthermore, when the brachial artery flow-mediated dilatation response was expressed relative to the shear stress

342 stimulus (which was also lower in South Asians), no between group differences were
343 observed.

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346

347 **GRANT AND DISCLOSURES**

348 None.

349 **REFERENCES**

- 350 1. **Al-Khazraji BK, Shoemaker LN, Gati JS, Szekeres T, and Shoemaker JK.**
351 Reactivity of larger intracranial arteries using 7 T MRI in young adults. *J Cereb Blood Flow*
352 *Metab* 39: 1204-1214, 2019.
- 353 2. **Atkinson G, and Batterham AM.** Allometric scaling of diameter change in the
354 original flow-mediated dilation protocol. *Atherosclerosis* 226: 425-427, 2013.
- 355 3. **Balarajan R.** Ethnicity and variations in the nation's health. *Health Trends* 27: 114-
356 119, 1995.
- 357 4. **Bathula R, Hughes AD, Panerai RB, Potter JF, Mc GTSA, Tillin T, Shore AC,**
358 **Hale R, Chambers J, Kooner J, and Chaturvedi N.** South Asians have adverse
359 cerebrovascular haemodynamics, despite equivalent blood pressure, compared with
360 Europeans. This is due to their greater hyperglycaemia. *Int J Epidemiol* 40: 1490-1498, 2011.
- 361 5. **Buchanan JE, and Phillis JW.** The role of nitric oxide in the regulation of cerebral
362 blood flow. *Brain Res* 610: 248-255, 1993.
- 363 6. **Chambers JC, McGregor A, Jean-Marie J, and Kooner JS.** Abnormalities of
364 vascular endothelial function may contribute to increased coronary heart disease risk in UK
365 Indian Asians. *Heart* 81: 501-504, 1999.
- 366 7. **Cho SJ, Sohn YH, Kim GW, and Kim J-S.** Blood flow velocity changes in the
367 middle cerebral artery as an index of the chronicity of hypertension. *J Neurol Sci* 150: 77-80,
368 1997.
- 369 8. **Clark JM, Skolnick BE, Gelfand R, Farber RE, Stierheim M, Stevens WC, Beck**
370 **Jr G, and Lambertsen CJ.** Relationship of 133Xe cerebral blood flow to middle cerebral
371 arterial flow velocity in men at rest. *J Cereb Blood Flow Metab* 16: 1255-1262, 1996.
- 372 9. **Coverdale NS, Gati JS, Opalevych O, Perrotta A, and Shoemaker JK.** Cerebral
373 blood flow velocity underestimates cerebral blood flow during modest hypercapnia and
374 hypocapnia. *J Appl Physiol* 117: 1090-1096, 2014.
- 375 10. **Fierstra J, Sobczyk O, Battisti-Charbonney A, Mandell DM, Poublanc J,**
376 **Crawley AP, Mikulis DJ, Duffin J, and Fisher JA.** Measuring cerebrovascular reactivity:
377 what stimulus to use? *J Physiol* 591: 5809-5821, 2013.
- 378 11. **Gemignani V, Faita F, Ghiadoni L, Poggianti E, and Demi M.** A system for real-
379 time measurement of the brachial artery diameter in B-mode ultrasound images. *IEEE Trans*
380 *Med Imaging* 26: 393-404, 2007.
- 381 12. **Gunarathne A, Patel JV, Gammon B, Gill PS, Hughes EA, and Lip GY.** Ischemic
382 stroke in South Asians: a review of the epidemiology, pathophysiology, and ethnicity-related
383 clinical features. *Stroke* 40: e415-423, 2009.
- 384 13. **Haight TJ, Bryan RN, Erus G, Davatzikos C, Jacobs DR, D'Esposito M, Lewis**
385 **CE, and Launer LJ.** Vascular risk factors, cerebrovascular reactivity, and the default-mode
386 brain network. *Neuroimage* 115: 7-16, 2015.
- 387 14. **Hainsworth AH, Oommen AT, and Bridges LR.** Endothelial cells and human
388 cerebral small vessel disease. *Brain Pathol* 25: 44-50, 2015.
- 389 15. **Hurr C, Kim K, Harrison ML, and Brothers RM.** Attenuated cerebral vasodilatory
390 capacity in response to hypercapnia in college-aged African Americans. *Exp Physiol* 100: 35-
391 43, 2015.
- 392 16. **Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, and**
393 **Luscher TF.** Nitric oxide is responsible for flow-dependent dilatation of human peripheral
394 conduit arteries in vivo. *Circulation* 91: 1314-1319, 1995.
- 395 17. **Junejo RT, Braz ID, Lucas SJ, van Lieshout JJ, Phillips AA, Lip GY, and Fisher**
396 **JP.** Neurovascular coupling and cerebral autoregulation in atrial fibrillation. *J Cereb Blood*
397 *Flow Metab* 0: 0271678X19870770, 2019.

18. **Junejo RT, Braz ID, Lucas SJE, van Lieshout JJ, Lip GYH, and Fisher JP.** Impaired Cerebrovascular Reactivity in Patients With Atrial Fibrillation. *J Am Coll Cardiol* 73: 1230-1232, 2019.
19. **Kety SS, and Schmidt CF.** The Effects of Altered Arterial Tensions of Carbon Dioxide and Oxygen on Cerebral Blood Flow and Cerebral Oxygen Consumption of Normal Young Men. *J Clin Invest* 27: 484-492, 1948.
20. **Khan NA, McAlister FA, Pilote L, Palepu A, Quan H, Hill MD, Fang J, and Kapral MK.** Temporal trends in stroke incidence in South Asian, Chinese and white patients: A population based analysis. *PLoS One* 12: e0175556, 2017.
21. **Lavi S, Gaitini D, Milloul V, and Jacob G.** Impaired cerebral CO₂ vasoreactivity: association with endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 291: H1856-1861, 2006.
22. **Liu P, De Vis JB, and Lu H.** Cerebrovascular reactivity (CVR) MRI with CO₂ challenge: A technical review. *Neuroimage* 187: 104-115, 2019.
23. **Markus H, and Cullinane M.** Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. *Brain* 124: 457-467, 2001.
24. **McKeigue PM, Ferrie JE, Pierpoint T, and Marmot MG.** Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. *Circulation* 87: 152-161, 1993.
25. **McKeigue PM, Miller GJ, and Marmot MG.** Coronary heart disease in south Asians overseas: a review. *J Clin Epidemiol* 42: 597-609, 1989.
26. **McKeigue PM, Shah B, and Marmot MG.** Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 337: 382-386, 1991.
27. **McSwain SD, Hamel DS, Smith PB, Gentile MA, Srinivasan S, Meliones JN, and Cheifetz IM.** End-tidal and arterial carbon dioxide measurements correlate across all levels of physiologic dead space. *Respir Care* 55: 288-293, 2010.
28. **Miller KB, Howery AJ, Rivera-Rivera LA, Johnson SC, Rowley HA, Wieben O, and Barnes JN.** Age-Related Reductions in Cerebrovascular Reactivity Using 4D Flow MRI. *Front Aging Neurosci* 11: 2019.
29. **Moghaddasi M, Mamarabadi M, and Habibi AH.** A comparison of cerebral vasomotor reactivity in diabetic and nondiabetic Iranian patients. *J Res Med Sci* 15: 50-53, 2010.
30. **Murphy C, Kanaganayagam GS, Jiang B, Chowienczyk PJ, Zbinden R, Saha M, Rahman S, Shah AM, Marber MS, and Kearney MT.** Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy UK South Asian men. *Arterioscler Thromb Vasc Biol* 27: 936-942, 2007.
31. **Nowak-Flück D, Ainslie PN, Bain AR, Ahmed A, Wildfong KW, Morris LE, Phillips AA, and Fisher JP.** Effect of healthy aging on cerebral blood flow, CO₂ reactivity and neurovascular coupling during exercise. *J Appl Physiol* 125: 1917-1930, 2018.
32. **Padilla J, Johnson BD, Newcomer SC, Wilhite DP, Mickleborough TD, Fly AD, Mather KJ, and Wallace JP.** Normalization of flow-mediated dilation to shear stress area under the curve eliminates the impact of variable hyperemic stimulus. *Cardiovasc Ultrasound* 6: 44, 2008.
33. **Peebles K, Celi L, McGrattan K, Murrell C, Thomas K, and Ainslie PN.** Human cerebrovascular and ventilatory CO₂ reactivity to end-tidal, arterial and internal jugular vein PCO₂. *J Physiol* 584: 347-357, 2007.
34. **Perregaux D, Chaudhuri A, Rao S, Airen A, Wilson M, Sung BH, and Dandona P.** Brachial vascular reactivity in blacks. *Hypertension* 36: 866-871, 2000.

35. **Poeppel T, Terborg C, Hautzel H, Herzog H, Witte O, Mueller H-W, and Krause B.** Cerebral haemodynamics during hypo- and hypercapnia. *Nuklearmedizin* 46: 93-100, 2007.
36. **Portegies ML, de Bruijn RF, Hofman A, Koudstaal PJ, and Ikram MA.** Cerebral vasomotor reactivity and risk of mortality: the Rotterdam Study. *Stroke* 45: 42-47, 2014.
37. **Rambihar VS, Rambihar SP, and Rambihar VS.** Race, ethnicity, and heart disease: A challenge for cardiology for the 21st century. *Am Heart J* 159: 1-14, 2010.
38. **Robbins PA, Conway J, Cunningham DA, Khamnei S, and Paterson DJ.** A comparison of indirect methods for continuous estimation of arterial PCO₂ in men. *J Appl Physiol* (1985) 68: 1727-1731, 1990.
39. **Sato K, Sadamoto T, Hirasawa A, Oue A, Subudhi AW, Miyazawa T, and Ogoh S.** Differential blood flow responses to CO₂ in human internal and external carotid and vertebral arteries. *J Physiol* 590: 3277-3290, 2012.
40. **Schmetterer L, Findl O, Strenn K, Graselli U, Kastner J, Eichler HG, and Wolzt M.** Role of NO in the O₂ and CO₂ responsiveness of cerebral and ocular circulation in humans. *Am J Physiol* 273: R2005-2012, 1997.
41. **Smolinski L, and Czlonkowska A.** Cerebral vasomotor reactivity in neurodegenerative diseases. *Neurol Neurochir Pol* 50: 455-462, 2016.
42. **Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, and Green DJ.** Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol* 300: H2, 2011.
43. **Toda N, Ayajiki K, and Okamura T.** Cerebral blood flow regulation by nitric oxide: recent advances. *Pharmacol Rev* 61: 62-97, 2009.
44. **Wild SH, Fischbacher C, Brock A, Griffiths C, and Bhopal R.** Mortality from all causes and circulatory disease by country of birth in England and Wales 2001-2003. *J Public Health (Oxf)* 29: 191-198, 2007.
45. **Zimmermann C, and Haberl RL.** L-arginine improves diminished cerebral CO₂ reactivity in patients. *Stroke* 34: 643-647, 2003.
46. **Zlokovic BV.** Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci* 12: 723-738, 2011.

479 **TABLES**

480 **Table 1.** Participant characteristics.

	Caucasian European	South Asian	P value
n	18	16	
Age (years)	21 [20-22]	21 [20-25]	0.505
Height (cm)	1.80 (0.07)	1.76 (0.06)	0.074
Weight (kg)	75.0 (8.5)	76.1 (11.4)	0.733
BMI (kg/m ²)	23.2 (2.4)	24.7 (3.2)	0.139
Waist circumference (cm)	78 [77-81]	80 [75-89]	0.387
Hip circumference (cm)	97 [95-98]	99 [93-100]	0.341
Waist / Height ratio (au)	0.44 (0.03)	0.47 (0.06)	0.050
Waist / Hip ratio (au)	0.80 [0.79-0.84]	0.82 [0.78-0.86]	0.557
Heart rate (b·min ⁻¹)	63 [58-66]	67 [58-73]	0.248
Systolic BP (mmHg)	124 (9)	119 (9)	0.070
Diastolic BP (mmHg)	67 [63-71]	67 [64-72]	0.972
Respiratory rate (b·min ⁻¹)	14 [13-15]	15 [13-16]	0.343

481

482 Values are displayed as mean (SD) when normally distributed or median [interquartile range]
 483 when non-normally distributed. BMI, body mass index; au, arbitrary units.

484

485 **Table 2.** Cerebrovascular CO₂ reactivity parameters.

		Caucasian	European	South Asian	Effect Size	P value
MCA V_m % (%·mmHg⁻¹)	Linear Slope	3.06 [2.77-3.18]		3.26 [2.83-3.45]	0.064	0.221
	R ²	0.96 (0.02)		0.95 (0.04)	0.316	0.397
	Exponent	0.029 [0.027-0.031]		0.030 [0.028-0.032]	0.126	0.691
	R ²	0.99 [0.97-0.99]		0.98 [0.96-0.99]	0.500	0.221
CVCi % (%·mmHg⁻¹)	Linear Slope	2.62 [2.26-2.83]		2.87 [2.66-3.29]	0.234	0.076
	R ²	0.96 [0.93-0.98]		0.94 [0.92-0.98]	0.250	0.458
	Exponent	0.027 [0.024-0.028]		0.028 [0.025-0.029]	0.105	0.629
	R ²	0.96 [0.94-0.98]		0.96 [0.93-0.97]	0.123	0.605
Hypercapnic MCA V_m Slope (cm·s⁻¹·mmHg⁻¹)	BL to 4%	1.49 [1.34-2.32]		1.84 [1.23-2.24]	0.083	0.931
	4% – 7%	2.61 (0.81)		2.53 (0.76)	0.102	0.754
Hypocapnic MCA V_m Slope (cm·s⁻¹·mmHg⁻¹)	BL to -4%	1.83 (1.10)		1.97 (1.03)	0.131	0.849
	-4% – -7%	1.00 (0.57)		0.92 (0.53)	0.145	0.656
Hypercapnic CVCi Slope (cm·s⁻¹·mmHg⁻²)	BL to 4%	0.017 (0.014)		0.022 (0.013)	0.370	0.282
	4% to 7%	0.023 (0.009)		0.022 (0.011)	0.099	0.769
Hypocapnic CVCi Slope (cm·s⁻¹·mmHg⁻²)	BL to -4%	0.025 (0.017)		0.024 (0.014)	0.064	0.934
	-4% to -7%	0.008 [0.005-0.014]		0.010 [0.002-0.014]	0.124	0.617
Hypercapnic %Δ MCA V_m /Δ P_{ET}CO₂ (%·mmHg⁻¹)	BL to 4%	3.02 [2.07-3.74]		3.06 [2.31-4.12]	0.079	0.666
	4% to 7%	3.57 [3.24-4.00]		3.68 [3.22-4.03]	0.072	0.986

Hypocapnic %Δ MCA V_m /Δ P_{ET}CO₂ (%·mmHg⁻¹)	BL to -4%	3.37 (1.69)	3.35 (1.64)	0.012	0.979
	-4% to -7%	1.99 (0.67)	1.87 (0.87)	0.154	0.662
Hypercapnic %Δ CVCi /Δ P_{ET}CO₂ (%·mmHg⁻¹)	BL to 4%	2.36 (1.92)	3.28 (2.08)	0.460	0.186
	4% to 7%	2.92 (1.27)	2.65 (1.46)	0.197	0.572
Hypocapnic %Δ CVCi /Δ P_{ET}CO₂ (%·mmHg⁻¹)	BL to -4%	3.51 (2.14)	3.53 (2.06)	0.009	0.969
	-4% to -7%	1.82 (1.17)	1.55 (1.33)	0.215	0.529

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487 Values are displayed as mean (SD) when normally distributed or median [interquartile range] when non-normally distributed. BL, baseline; R²,
488 coefficient of determination; 4%, first hypercapnic step containing 4% CO₂; 7%, second hypercapnic step containing 7% CO₂; -4%, first
489 hypocapnic step intended to produce an equal and opposite change in P_{ET}CO₂ as observed with 4% CO₂; -7%, second hypocapnic step intended
490 to produce an equal and opposite change in P_{ET}CO₂ as observed with 7% CO₂.

491

492 **Table 3.** Flow-mediated dilatation parameters in Caucasian Europeans and South Asians.

	Caucasian European	South Asian	Effect Size	P value
Baseline diameter (mm)	4.13 [3.83-4.37]	4.30 [4.03-4.51]	0.194	0.285
Baseline velocity (cm.s ⁻¹)	11.82 [8.65-20.29]	13.11 [11.28-29.65]	0.579	0.208
Baseline blood flow (ml.min ⁻¹)	49.32 [37.01-75.30]	59.31 [42.07-143.41]	0.632	0.196
Peak diameter (mm)	4.47 [4.14-4.68]	4.55 [4.29-4.79]	0.011	0.666
Peak blood flow (ml.min ⁻¹)	363.37 (108.93)	339.88 (128.06)	0.197	0.567
Time to peak flow (s)	12.50 [11.00-14.75]	11.50 [9.75-13.50]	0.047	0.404
Absolute FMD (mm)	0.31 (0.09)	0.23 (0.13)	0.715	0.062
Time to peak diameter (s)	68.28 (27.24)	66.56 (24.72)	0.066	0.849
FMD_C (%)	7.39 (2.28)	5.51 (2.94)	0.715	0.044
SR_{AUC} (s ⁻¹)	19028.11 (8991.70)	12519.81 (5091.05)	0.891	0.016

493

494 Values are displayed as mean (SD) when normally distributed or median [interquartile range]

495 when non-normally distributed. FMD, flow-mediated dilatation; FMD_C, corrected flow-

496 mediated dilatation; SR_{AUC}, shear rate area under the curve.

497

FIGURE LEGENDS

Figure 1. Baseline MCA V_m , CVCi, MAP and $P_{ET}CO_2$ in Caucasian Europeans and South Asians. MCA V_m , middle cerebral artery mean flow velocity; CVCi, cerebrovascular conductance index; MAP, mean arterial pressure; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide. Data expressed as individual values and means with SD. * represents $P < 0.05$.

Figure 2. MCA V_m , CVCi and MAP responses to the cerebrovascular CO_2 reactivity protocol in Caucasian Europeans and South Asians. Symbols show mean and standard error of the mean.

Figure 3. Cerebrovascular CO_2 reactivity in Caucasian Europeans and South Asians. Cerebrovascular CO_2 reactivity is expressed as the slope of MCA V_m change in cm/s (Δ) (panel A) and Δ CVCi (panel B) versus $\Delta P_{ET}CO_2$ in mmHg. Horizontal bars show mean and SD.

Figure 4. Flow-mediated dilatation (FMD) in Caucasian Europeans and South Asians. FMD is expressed as a percentage change (panel A) and as a ratio between FMD (%) and SR_{AUC} (panel B). Horizontal bars show mean and SD.







